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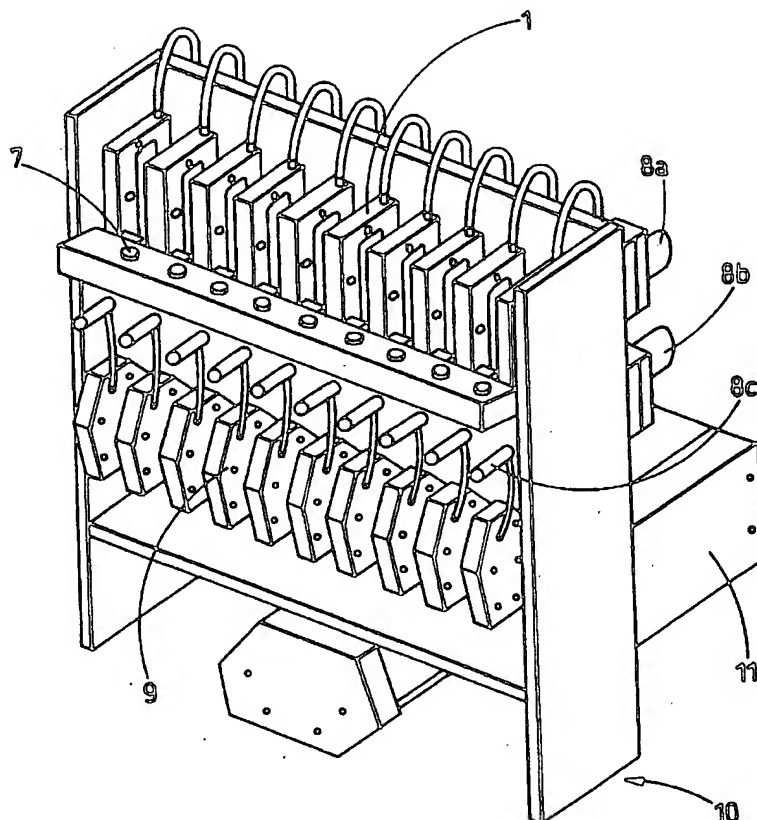
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(54) Title: MODULAR AUTOMATED SAMPLE PROCESSING APPARATUS



(57) Abstract: An automated sample processing apparatus comprising a plurality of support blocks each adapted to receive a solid support bearing the sample to be processed and/or reagent, such that in use the support block and the solid support form a substantially enclosed chamber, means for introducing fluid into the chambers and removing fluid from the chambers, and means for heating the chambers and/or the fluid; and a method of processing samples using this apparatus.

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ning of each regular issue of the PCT Gazette.*

## MODULAR AUTOMATED SAMPLE PROCESSING APPARATUS

The present invention relates to a method for processing samples, particularly biological samples on solid supports such as microarrays of genes, and to apparatus  
5 adapted for use in this method.

Genomics, particularly in the area of gene expression, is used increasingly in the pharmaceutical and biotechnology sector. Rapid technological developments have enabled gene expression monitoring, see Brown *et al*, *Nature Genetics*, 1999, 21, 33-37; Watson *et al*, *Current opinion in Biotechnology*, 1998, 9, 609-614; and Bowtell *et al*,  
10 *Nature Genetics supplement*, 1999, 21, 25-32, notably using microarrays. The level of expression of thousands of genes in one sample can be measured at one time, see Marton *et al*, *Nature Medicine*, 1998, 4, 11, 293-1301; De Risi *et al*, *Science*, 1997, 278, 680-686; and Lashkari *et al*, 1997, 94, 3057-13062. The use of microarrays and their potential for screening has been identified, see DeBouck *et al*, *Nature*, 1999, yet the automation of  
15 their use leaves a lot to be desired. There is currently no commercial system which will automate the process from sample loading right through to the stage where a microarray which can be imaged is obtained. The initial stages of making microarrays, by stamping onto grids has been automated, with a number of manufacturers producing commercially available "gridding" machines. The final steps of using microarrays have also received  
20 attention and there are a number of imaging instruments on the market, some with carousels which can automatically load a number of slides for imaging. There are however a number of steps, particularly the steps of sample loading and hybridisation, and also considerable operator intervention, which must be automated if microarrays are to be exploited for use in a high throughput system. One commercial system available on  
25 the market which endeavours to provide automation of these stages is the Genomics Solutions' GeneTAC™ hybridisation station. However, this system has a number of drawbacks:

it is only available for 2 slides at any one time;

there are only 3 positions for solutions, which limits the number of washes  
30 which can be performed, and means that only the hybridisation step, and not the slide preparation step, can be automated;

the processing solutions and sample are heated whilst in contact with the slide;

the sample injection step is manual; and  
the system is based on a vacuum which prevents even removal of the  
solutions, resulting in streaks and preventing the slides from being read accurately.

The present invention relates to an apparatus which allows the automation of slide  
5 preparation, sample preparation, sample loading, hybridisation, washing and drying; and  
to a method of processing samples using this apparatus. The apparatus and the method of  
the invention are convenient to use and give reproducibly high quality microarrays. A  
particularly surprising finding is that the apparatus and method of the invention  
significantly reduces or eliminates the phenomenon of "streaking", whereby a portion of  
10 the sample that is not firmly adhered to the solid support may spread beyond the bounds  
of the original spots, commonly seen in prior art methods. A further significant advantage  
over prior art methods is the reproducibility of the resultant microarrays, improved  
reproducibility arises largely because of the greater consistency in timing of the  
processing steps compared to a manual method.

15 Further advantages of the method of the invention over known methods for the  
processing of samples, include:

the system is self-contained so that there is minimum handling of the samples,  
thus reducing levels of artefact. Once the sample is inserted into the chamber it is not  
disassembled again until after the processing is complete;

20 there are no problems with either the sample "drying out" at high temperature or  
interference of sealant with the processing, e.g. the hybridisation reaction, or  
contaminating the sample; and

processing solutions are agitated giving even processing, e.g. hybridisation,  
resulting in higher reproducibility between replicates on the same sample and, in turn  
25 higher reproducibility between replicate samples.

Thus according to the invention there is provided an automated sample  
processing apparatus comprising:

a plurality of support blocks each adapted to receive a solid support bearing the  
sample to be processed and/or reagent, such that in use the support block and the solid  
30 support form a substantially enclosed chamber;

means for introducing fluid into the chambers and removing fluid from the  
chambers; and

means for heating the chambers and/or the fluid.

According to a further aspect of the invention there is provided a method for automatically processing a sample comprising:

5 inserting a solid support bearing a sample to be processed and/or reagent into one of a plurality of support blocks such that the support block and the solid support form a substantially enclosed chamber; and

processing a sample within the chamber using a predetermined protocol comprising steps involving the introduction of fluid into the chamber, the removal of fluid from the chamber, and the heating of the chamber and/or the fluid.

10 In the apparatus and method of the invention the solid support bearing the sample to be processed and/or reagent is preferably in the form of a solid support bearing an immobilised material which is capable of interacting with a fluid introduced into the chamber. The immobilised material may comprise DNA, protein or protein recognition units e.g. antibodies or complementarity determining regions, in which case the solid support preferably takes the form of a microarray. The immobilised material may also be a  
15 tissue section. The fluid which is introduced into the chamber and which is capable of interacting with the immobilised material may contain a biological sample e.g. a tissue or a cell extract. Substances present in the biological sample will preferably bind or hybridise to the immobilised material. Binding or hybridisation may be visualised in a processed by  
20 staining or other methods such as radiolabelling or chemical labelling, either before or after the binding step. Biotinylation, fluorescence, chemiluminescence or other labelling techniques may also be used. Analysis of the resultant binding pattern may be undertaken using an appropriate detection system such as optical or laser scanning technology and a comparison of results between, for example, drug treated and untreated control tissues can  
25 be undertaken using suitable computer software.

A biological sample to be processed using the method of the invention will preferably be from a mammal treated with a chemical compound of therapeutic potential. The method of the invention provides a fast, reproducible and robust methods for determining e.g. nucleotide or protein expression pattern of tissues or cell types, i.e.  
30 genomics or proteomics. The method may be applied in, for example, toxicology, clinical trials, process monitoring, biotechnology and pharmaceuticals, either as a research or diagnostic tool. The method may be used for analyzing the spatial and temporal changes

in the expression of proteins in mammalian tissues and cell types when exposed to environmental stimuli such as pharmaceutical reagents, as well as other environmental stimuli or factors. The method allows for the comparison of targets treated with therapeutic drugs and untreated controls to provide a quantitative and/or semi-qualitative analysis of treated and untreated (control) target systems e.g. cells, tissues or biological organisms. The method may also be adapted for use in, for example, drug manufacturing, purity analysis (e.g. in food and agriculture) and for diagnostics, e.g. for detecting proteins in urine or blood.

10 In a preferred aspect, the invention comprises a method for automatically processing a microarray comprising:

inserting a solid support bearing a microarray into one of a plurality of support blocks such that the support block and the solid support form a substantially enclosed chamber; and processing the microarray within the chamber using a predetermined protocol comprising steps involving:

15 heating the microarray;  
introducing fluid into the microarray comprising a biological sample capable of interacting with the microarray;  
removal of the fluid from the chamber;  
washing the microarray; and  
20 drying the microarray.

The apparatus of the invention may contain any number of support blocks from two upwards. The apparatus preferably comprises at least five support blocks. The support blocks may be present in, for example, banks of 5, 8 or 10 blocks. It will be readily apparent to those skilled in the art how further blocks may be added to the apparatus.

25 The support blocks used in the invention may wholly enclose the solid support, e.g. a glass slide, bearing the sample to be processed and/or reagent, i.e. the support block provides an enclosed, fluid tight container for the solid support. In this case the support block preferably comprises top and bottom members which may be separated, thus allowing easy insertion and removal of the solid support.

30 More preferably, in use, part of the internal surface of the substantially enclosed chamber is defined by the sample and/or reagent bearing surface of the solid support.

When the solid supports are made of transparent glass this has the advantage of allowing the inside of the chamber to be visible. In this case, once again, the support block preferably comprises top and bottom members which may be separated, thus allowing easy insertion and removal of the solid support.

- 5           When the support block comprises top and bottom members they preferably comprise sealing means to assist in the provision of an enclosed, fluid tight chamber. The sealing means may comprise clamping means such as screws. When clamping means are used to seal the chamber it is preferable that the pressure exerted by the clamping means is uniformly distributed over the support block to ensure an efficient seal between the two
- 10 members. The sealing of the block may be further assisted by the provision of a gasket, e.g. an O-ring, between the top and bottom members, or between one or both of the top and bottom members and the solid support, this gasket may be made of any resilient material that is inert to the processing conditions employed, e.g. silicon rubber.

- The support block is preferably substantially rectangular in cross section, however
- 15 other shapes may be employed depending on the configuration of the solid supports. The support blocks may be made from any substantially rigid material that is inert to the processing conditions employed, for examples metal or plastics.

- The means for introducing fluid into the chambers and removing fluid from the chambers preferably comprise a fluid inlet and a fluid outlet in the body of the sample
- 20 block. The fluid inlet and fluid outlet will be in contact with the interior of the chamber in use.

- The apparatus preferably comprises fluid storage means in connection with the means for introducing fluid into the chambers, the storage means being adapted to contain the fluid that is introduced into the chambers during processing. The fluid inlet is
- 25 preferably interchangeably connected to a plurality of fluid storage means thus allowing several fluids to be used in the processing of the sample. Fluids used in processing of the sample include liquids and gases.

- The apparatus also preferably contains means for directing fluid that is removed from the chamber to waste.

- 30           The configuration of the means for introducing fluid into the chambers and removing fluid from the chambers are preferably such that a fluid can be repeatedly circulated through the chamber. A means of circulating fluid over the solid support is



particularly advantageous when the processing involves the hybridisation of a sample to a microarray since it results in more uniform hybridisation than can be achieved when the fluid containing the hybridisation sample is static. The circulation of fluid through the chamber may continue for e.g. up to 24 hours, suitably for 2-16 hours. The rate of  
5 circulation of fluid is preferably controllable, fluid is preferably circulated slowly.

Fluid connections used in the apparatus of the invention, e.g. tubing connecting the means for introducing fluid into the chambers/removing fluid from the chambers and the fluid storage means/waste, is preferably narrow bore in order to minimise the quantity of reagent/samples/fluids that are required in the sample processing.

10 Fluids are preferably introduced and removed from the chambers by positive pressure, e.g. a pumping mechanism.

Fluids that are typically used in the process of the invention include hot water which is used to prepare solid supports, e.g. glass slides, solvents used for support preparation, samples, wash solutions and gases, e.g. air for drying solid supports received  
15 in the support blocks.

The solid supports are preferably dried in a manner which does not leave any residue or "streaks" on supports, such that e.g. in the processing of microarrays there is no interference with the imaging of the spots. The solid supports are preferably dried using a flow of gas e.g. air, the gas may be at an elevated temperature e.g. about 50°C. In use  
20 the support blocks and the means for introducing fluid into the chambers and removing fluid from the chambers are preferably maintained in a thermally controlled environment. This is particularly advantageous when the processing of the sample involves the circulation of sample/reagent through the chamber, since no cooling/heating of the sample will occur when it is outside the chamber. In addition it is preferable that fluids may be  
25 introduced into the chamber at a chosen, e.g. elevated temperature.

The apparatus is preferably adapted to allow easy cleaning such that there is no risk of contamination between different runs of the apparatus.

The solid supports preferably bear microarrays of reagents which are used in the processing of a sample, and the sample is introduced as a fluid into the chamber.

30 The various steps involved in the processing of a sample according to the invention can preferably be programmed e.g. as to time and temperature. Different support blocks in the apparatus according to the invention can preferably be controlled

independently thus allowing different sample processing protocols to be used simultaneously in a single apparatus.

The method of the invention is preferably performed under computer control. The computer control means may comprise a personal computer or may be integrated with the apparatus adapted to perform the claimed method. The computer control means  
5 preferably controls one or more of the following parameters: temperature, fluid volume and flow rate and timing of various stages in the sample processing.

A typical automated procedure which may be performed using the apparatus of the invention is as follows:

- 10 1. Insert slide into support chamber.
2. Flush through with isopropanol for a time between 1 and 10 min.
3. Wash slide for 2-5 min at a temperature not less than 70°C.
4. Raise the sample temperature to 100°C for a period of one minute before injection to the chamber.
- 15 5. Inject the sample and circulate it for a period between 2 and 16 hours at a temperature that is set between 42°C and 60°C.
6. Wash the prepared slide with two solutions consecutively twice for a period between 1 and 5 min for each slide at a temperature in the range 20°C - 60°C.
7. Air dry each slide with any gas for the same period 1-5 min or until there are no  
20 streaks left on the slide.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

25 The apparatus and method according to the invention are illustrated by the following examples and figures.

Figure 1 is a schematic representation of an apparatus according to the invention, comprising five support blocks and illustrating the status of fluid inlets and outlets during a sample processing protocol similar to that described above.

30 Figure 2 shows a plan view of the inner surface, end and cross-sectional views of the bottom member of a two member support block (1a) of the type wherein in use part of the internal surface of the substantially enclosed chamber is defined by the sample and/or

reagent bearing surface of the solid support. A fluid inlet and fluid outlet (2a, 2b) are shown together with a groove (3) for retaining a O-ring used to enhance the seal between the support block and solid support.

5 Figure 3 shows a plan view of the outer surface, a plan view of the inner surface and a cross-sectional view of the top member of a two member support block (1b) of the type wherein in use part of the internal surface of the substantially enclosed chamber is defined by the sample and/or reagent bearing surface of the solid support. The inner surface and the cross-section show a flange (4) adapted to retain the solid support. Four clamping screws (5) are illustrated in the view of the outer surface.

10 Figure 4 shows side and top views of a clamping screw for use with the support block of Figures 2 and 3.

Figure 5 shows a perspective view of the assembled support block (1) comprising bottom (1a) and top members (1b), and a solid support (6) held in position in the block. The O-ring which is not visible in this figure forms a seal between the sample and/or  
15 reagent bearing surface of the solid support and the inner surface of the bottom member of the support block (1a).

Figure 6 shows a photograph of a single assembled support block and solid support during the processing of a sample.

20 Figure 7 shows a perspective view of an apparatus according to the invention, comprising ten support blocks.

Figures 8, 9 and 10 are visual inspection images of microarrays produced in Example 1, viz: Figure 8 - 600µl, Without accelerator, control manual method; Figure 9 - 600µl, Without accelerator, automated method of invention; Figure 10 - 600µl, With accelerator, automated method of invention.

25 Figure 11 shows the correlation between the right hand side (RHS) and left hand side (LHS) of the 600µl without accelerator, control manual slide.

Figure 12 shows the correlation between the RHS and LHS of the 600µl without accelerator, automated method of the invention slide.

30 Figure 13 shows the correlation between the RHS and LHS of the 600µl with accelerator, automated method of the invention slide.

Figure 14 is a bar chart representing the solidity of the average spot as a percentage of the maximum solidity value for the three tests. Accompanying this bar chart is the relevant data.

5     Figure 15 is a bar chart representing the Roundness of the average spot as a percentage of the maximum Roundness value for the three tests. Accompanying this bar chart is the relevant data.

Figure 16 is a bar chart representing the Circularity of the average spot as a percentage of the maximum Circularity value for the three tests. Accompanying this bar chart is the relevant data.

10     Figure 17 is a bar chart representing the average Artefact levels of each spot. Accompanying this bar chart is the relevant data.

#### EXAMPLE 1

15     Comparison of results obtained from microarray hybridisations using the automated sample processing apparatus as illustrated in Figures 1 and 6 with those obtained using a manual method

Sufficient labelled cDNA was made up for 3 hybridisations on 3 different microarray slides. Two of these experiments were performed in the apparatus of the invention and the third was using control (manual) method. All hybridisations were carried out using  
20     the same hybridisation volume (600ul) and the same cDNA concentration. In one of the automatically controlled experiments accelerator was added in order to enhance the hybridisation kinetics.

#### METHOD

25     Preparation of labelled cDNA

Sufficient cDNA was reverse transcribed for all three experiments (10ug total RNA per hybridisation). The cDNA was made from rat liver and fluorescently labelled during the transcription reaction by a method similar to that described in DeRisi et al, 1997, Science, 278, 680-686, except that Cy3 was replaced with Alexa 546.

30

#### Hybridisation solution

The hybridisation solution was made up as follows:

labelled cDNA	100µl
2 x hybridisation buffer	300µl
distilled water	200µl

5

For the experiment with accelerator:

labelled cDNA	100µl
2 x hybridisation buffer	300µl
distilled water	100µl
accelerator	100µl

10

The hybridisation buffer used was 10xSSC, 0.2% SDS.

The accelerator used was 60% polyethylene glycol (PEG). BDH Laboratory Supplies.

15

#### Microarray slides

Microarrays of PCR amplified genes were obtained from the Biotechnology and Genetics Dept, SmithKline Beecham. These comprised 1248 genes, mainly from the IMAGE consortium (Research Genetics). These genes were spotted down on two sides of a glass slide (left hand side, LHS and right hand side, RHS) in 6 grids of 16 x 13 spots. Each side of the glass represents one replicate.

20

#### Slide preparation (for the manual method)

Prior to using the slides they must be rinsed in isopropanol and boiled to remove grease and debris. The slide for manual hybridisation was rinsed in isopropanol for 10 minutes and boiled in distilled water for 10 minutes. It was then assembled in the hybridisation chamber.

25

#### Hybridisation and wash conditions (manual method)

600ul of hybridisation solution (see above) was heated to 100°C for 3 minutes before pipetting into the hybridisation chamber (see figures). Using the manual method the chamber was sealed and rotated overnight in an incubator for 16 hours at 50°C. The slide

30

was then removed from the chamber and washed for 10 minutes in 2 x SSC (Sigma), 0.1% saline followed by a 5 minute wash in 0.1% SSC, 0.1% saline (both at 40°C). The slide was centrifuged in order to remove any traces of buffer.

5 Slide preparation, hybridisation and wash conditions (automated method)

Bottles of the appropriate reagents were replenished, including wash buffers (see above for composition) and boiling water. Freshly printed slides were assembled in hybridisation chambers on the support block of the automated machine. Sample was added to the sample block and the slides were automatically pre-rinsed, hybridised,

10 washed and dried according to the following protocol:

Instrument Timings:

	Hot Water Flush (mins):	6
15	Isopropanol Flush (mins):	6
	Hot Temperature Hold (mins):	1
	Sample Fill (secs):	30
	Circulate (hrs):	16
	Sample Empty (mins):	3
20	*Final Wash (mins):	5
	Air Dry (min):	3

\*The 'Final Wash' step incorporates two washes (low and high stringency) both run for the time stated. The temperature was kept at 50°C for the duration of the experiment, but  
25 the sample holding block was heated to 80-100°C before sample was applied to the slide.

Imaging and analysis

Imaging was carried out using a Molecular Dynamics Generation III Scanner (Amersham Pharmacia Biotech, UK) at 550-600nm. Images were analysed using a proprietary  
30 software package that provides data comprising:

spot volume (with and without dust/artefact)

spot circularity (a measure of how even the circumference of the spot is)

spot roundness (a measure of how misshapen the spot is)

spot solidity (a measure of how even the signal intensity is across the spot)

These criteria were examined in order to compare the total signal, the reproducibility of signal, the quality of hybridisation to spots and the amount of artefact in the

5 hybridisations.

## RESULTS

All slides demonstrated reasonable hybridisation of the probe to the spots indicating that the cDNA was transcribed and well labelled.

10

### Visual observations

A visual inspection of the results (see Figures 7-9) revealed the following:

- Slides hybridised in the automated equipment were dry when removed from the chambers
- 15 ◦ There was no streaking caused by drying in the automated station
- There was considerably less artefact when using the automated method
- There is more dynamic range (i.e. maximum/minimum signal) using the automated method
- The maximum signal intensity is greater when using the automated method

20

### Reproducibility

The spot volumes (without dust) were used for a comparison of left and right hand sides of the slides. If the duplicate spots on the two sides of the slide give a similar signal then the correlation is higher (i.e. closer to 1). Figures 10-12 show the correlations of left and right

25 hand sides of the slides for the manual method and the automated method (with and without accelerator). The correlations are 0.59, 0.76 and 0.95 respectively, indicating that the automated method increases the reproducibility of duplicate spots within a slide.

### Spot quality

30 Figures 13-15 show graphs of the roundness, circularity and solidity of spots. These criteria can be used to measure spot quality, since rounder, more circular and even spots facilitate image analysis and indicate even hybridisation. These criteria were measured for

each spot and then averaged for one side of a microarray slide. In all cases the automated method gave rise to more solid, more circular, rounder spots than the manual method. The addition of accelerator also improved these results.

5    Artefact

One problem arising in using microarrays is that large amounts of artefact (often dust) contaminate the slide. During image analysis these are often included as spot signals hence leading to erroneous results. Reducing artefact on the slide facilitates image analysis giving rise to "real" results and hence improves reproducibility. In this  
10    experiment the amount of artefact was calculated by measuring the "spot volume with dust" and the "spot volume without dust" (as measured by the software). The calculated volume of "dust" was averaged for all spots in a given side of the slide and the results are shown in Figure 16. As can clearly be seen using the automated method massively reduces the amount of artefact occurring on the slide. Again, using accelerator enhances the result.

15

CONCLUSIONS

The experiment demonstrated that the automated sample processing apparatus provides hands-off sample preparation, slide preparation, sample application, hybridisation, washing and drying. The processed slides have a low background and there is no smearing or  
20    streaking of wash solutions. The automatically processed slides are easier to analyse. This method of the invention therefore offers improvements in time, labour, reproducibility, quality and accuracy of microarray experiments when compared to manual methods of sample processing.



## Claims:

1. An automated sample processing apparatus comprising:  
a plurality of support blocks each adapted to receive a solid support bearing the  
5 sample to be processed and/or reagent, such that in use the support block and the solid  
support form a substantially enclosed chamber;  
means for introducing fluid into the chambers and removing fluid from the  
chambers; and  
means for heating the chambers and/or the fluid.  
10
2. The apparatus according to claim 1 which comprises at least five support blocks.
3. The apparatus according to claim 1 or 2 wherein, in use, part of the internal  
surface of the substantially enclosed chamber is defined by the sample and/or reagent  
15 bearing surface of the solid support.
4. The apparatus according to any one of the preceding claims wherein the support  
block comprises top and bottom members which may be separated to allow insertion and  
removal of the solid support.  
20
5. The apparatus according to any one of the preceding claims wherein the means for  
introducing fluid into the chambers and removing fluid from the chambers comprises a  
fluid inlet and a fluid outlet in the body of the sample block which are in contact with the  
interior of the chamber in use.  
25
6. The apparatus according to any one of the preceding claims which additionally  
comprises fluid storage means in connection with the means for introducing fluid into the  
chambers.
- 30 7. The apparatus according to any one of the preceding claims wherein the  
configuration of the means for introducing fluid into the chambers and removing fluid  
from the chambers are such that a fluid can be repeatedly circulated through the chamber.

8. The apparatus according to any one of the preceding claims wherein fluids are introduced and removed from the chambers by positive pressure.
- 5 9. The apparatus according to any one of the preceding claims wherein the support blocks and the means for introducing fluid into the chambers and removing fluid from the chambers are maintained in a thermally controlled environment in use.
- 10 10. The apparatus according to any one of the preceding claims wherein fluids may be introduced into the chamber at an elevated temperature.
11. The apparatus according to any one of the preceding claims wherein the solid supports are dried using a flow of gas.
- 15 12. The apparatus according to any one of the preceding claims wherein the solid supports bear microarrays.
13. A method for automatically processing a sample using the apparatus defined in any one of the preceding claims.
- 20 14. A method for automatically processing a sample comprising:  
inserting a solid support bearing a sample to be processed and/or reagent into one of a plurality of support blocks such that the support block and the solid support form a substantially enclosed chamber; and  
25 processing a sample within the chamber using a predetermined protocol comprising at least steps involving the introduction of fluid into the chambers, the removal of fluid from the chambers, and the heating of the chamber and/or the fluid.
15. A method for automatically processing a microarray comprising:  
30 inserting a solid support bearing a microarray into one of a plurality of support blocks such that the support block and the solid support form a substantially enclosed

chamber; and processing the microarray within the chamber using a predetermined protocol comprising steps involving:

- heating the microarray;
  - introducing fluid into the microarray comprising a biological sample capable of
  - 5 interacting with the microarray;
  - removal of the fluid from the chamber;
  - washing the microarray; and
  - drying the microarray.
- 10 16. The method according to any one of claims 13 to 15 which is performed under computer control.

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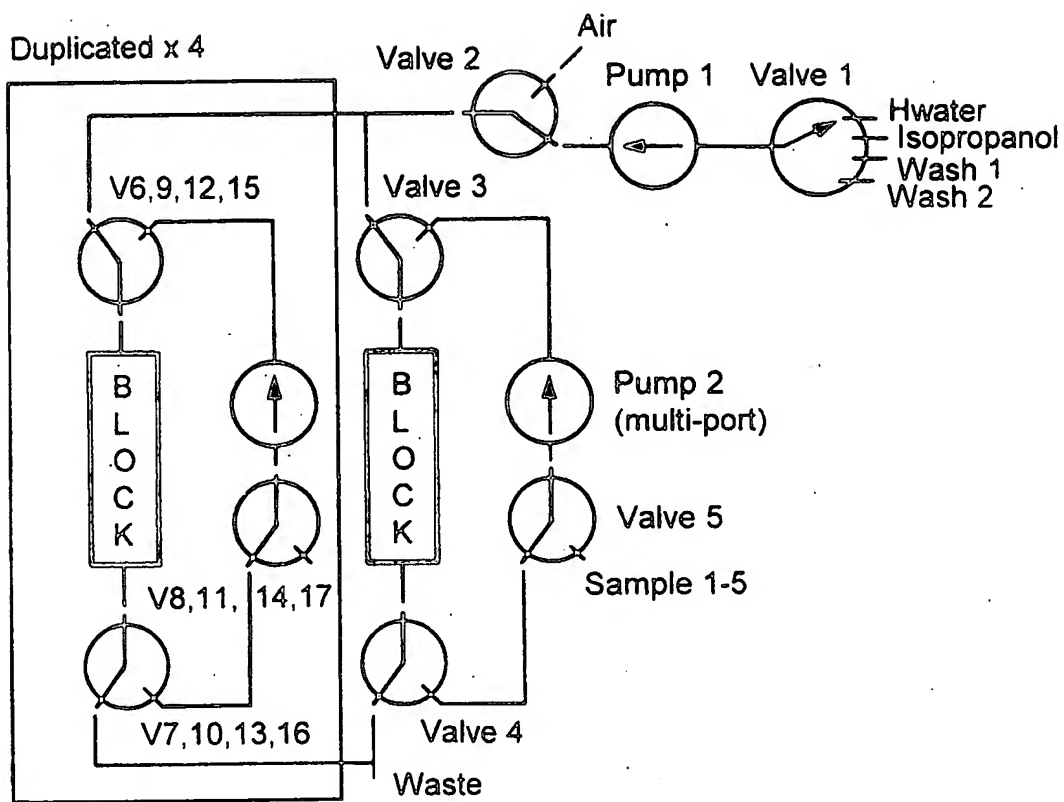


Fig. 1

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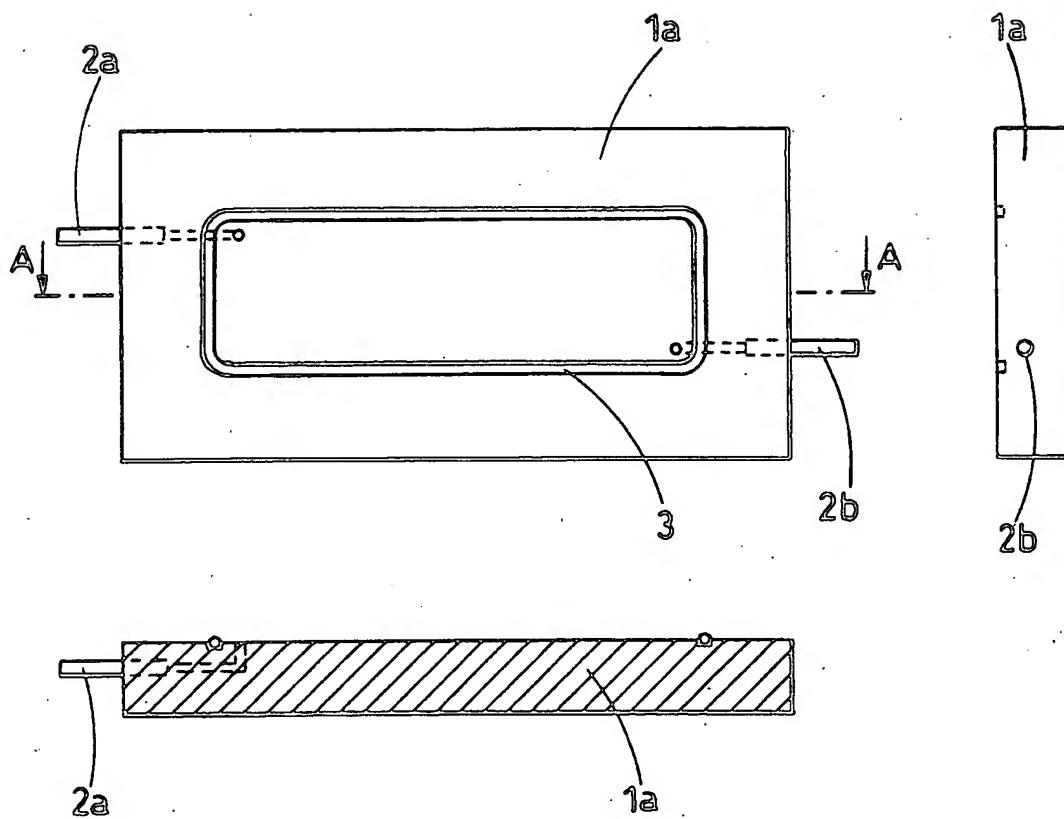


Fig. 2

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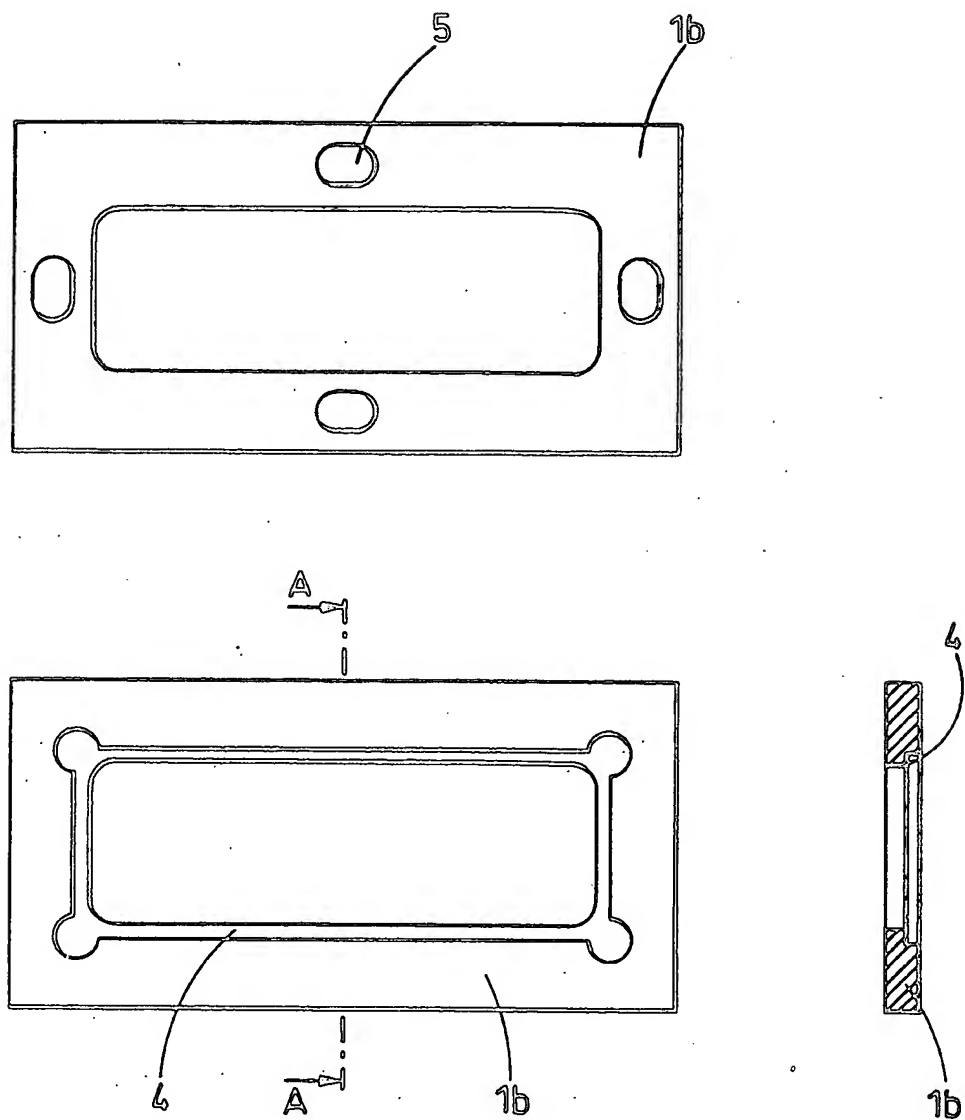


Fig. 3

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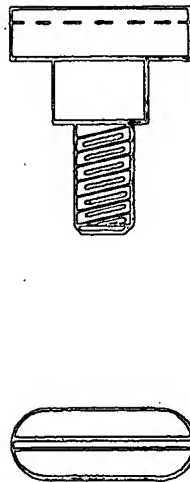


Fig. 4

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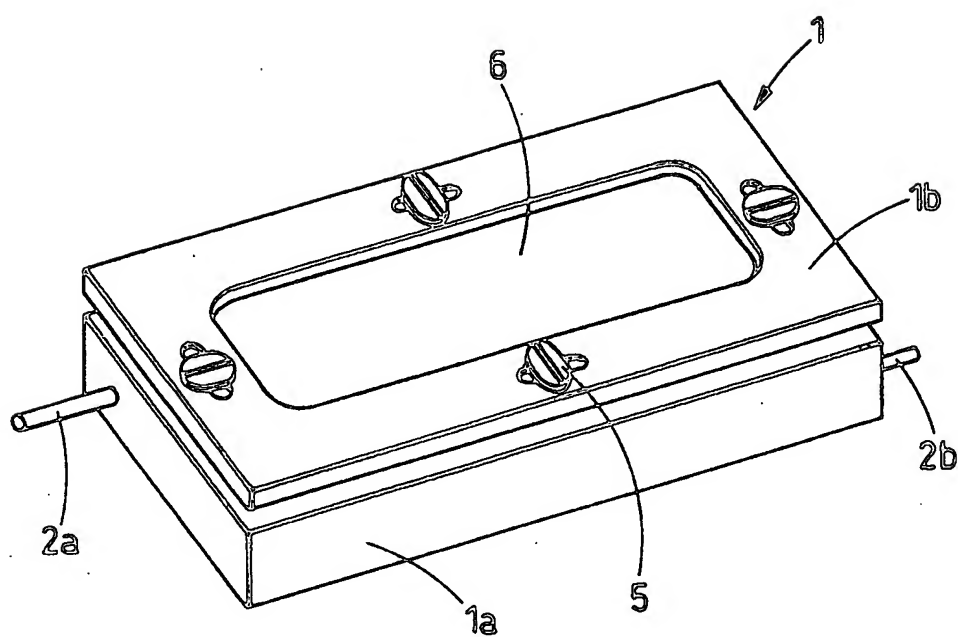


Fig. 5



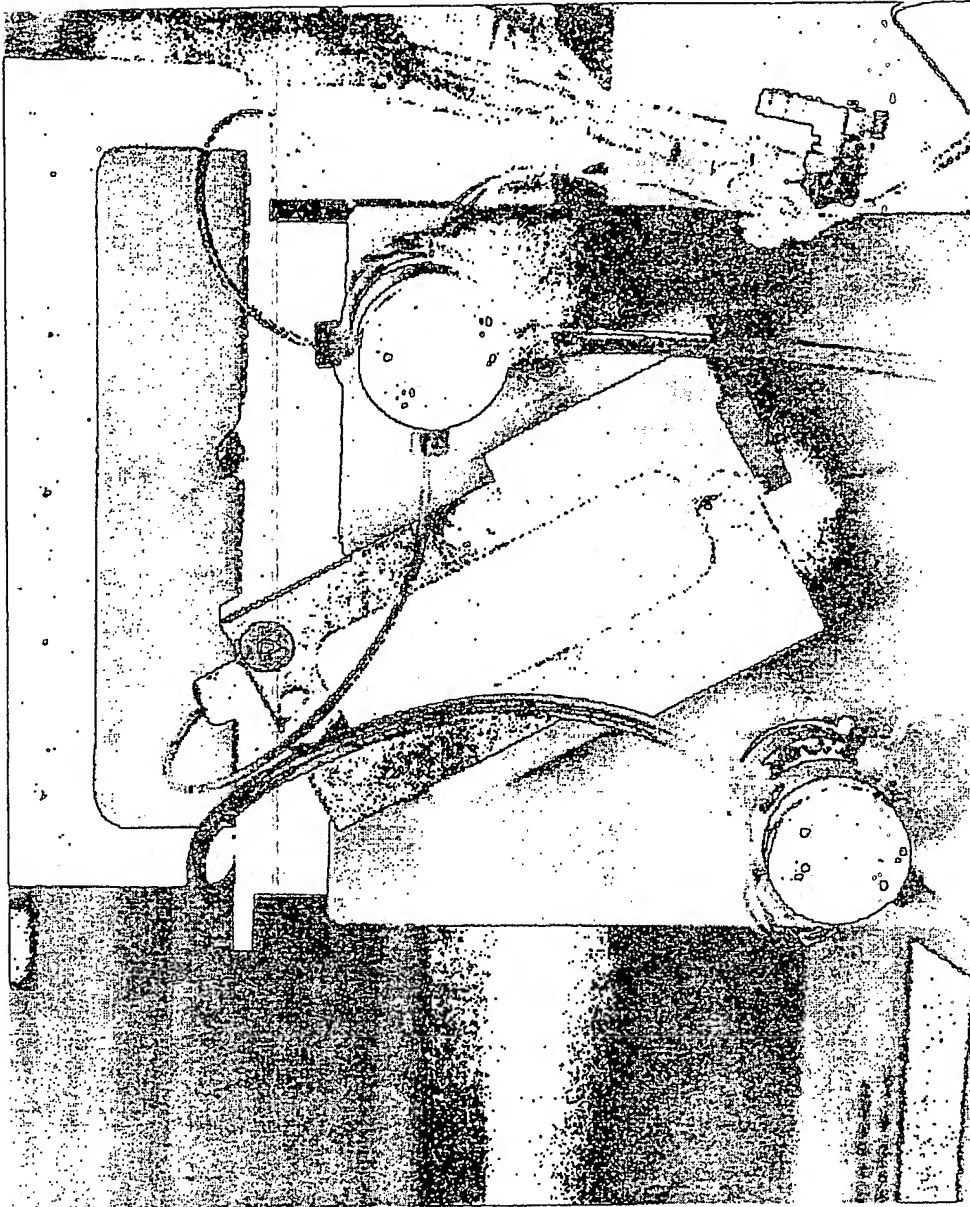


Fig. 6

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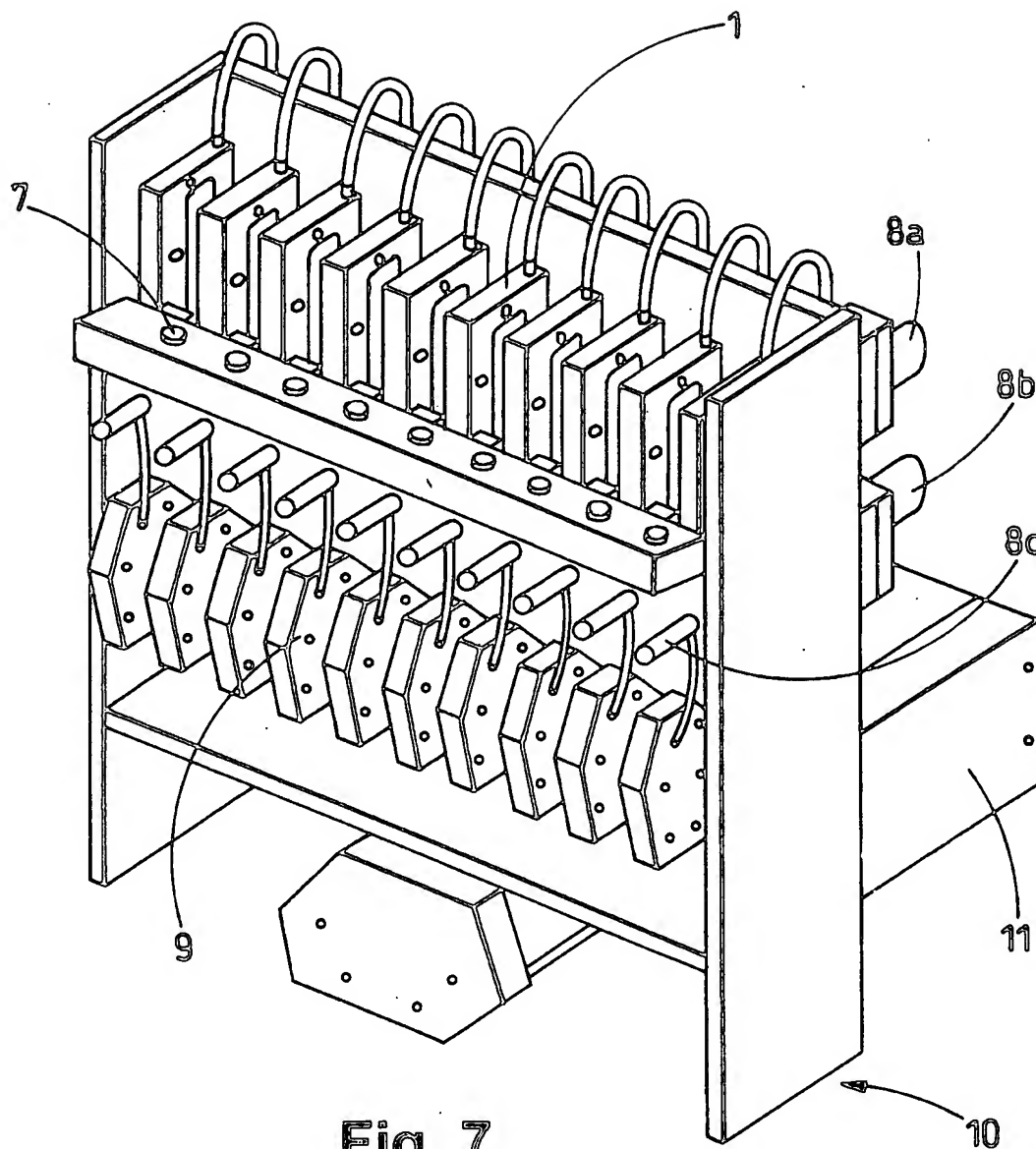


Fig. 7

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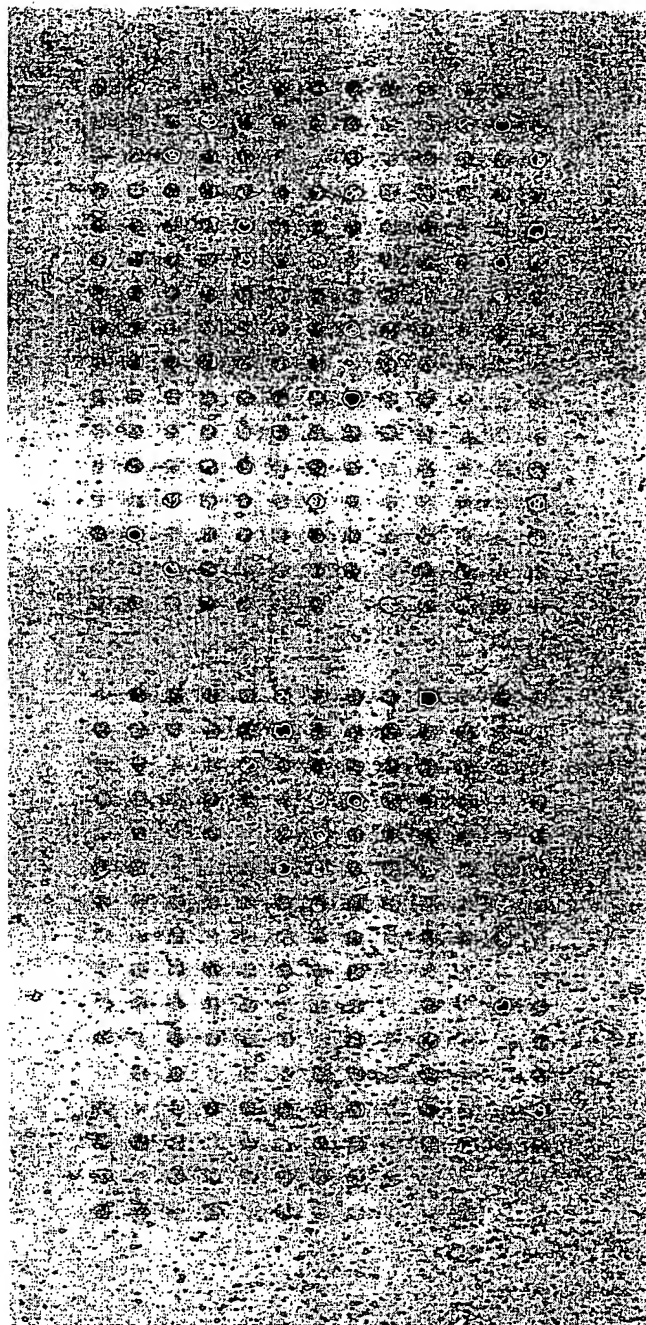


Fig. 8

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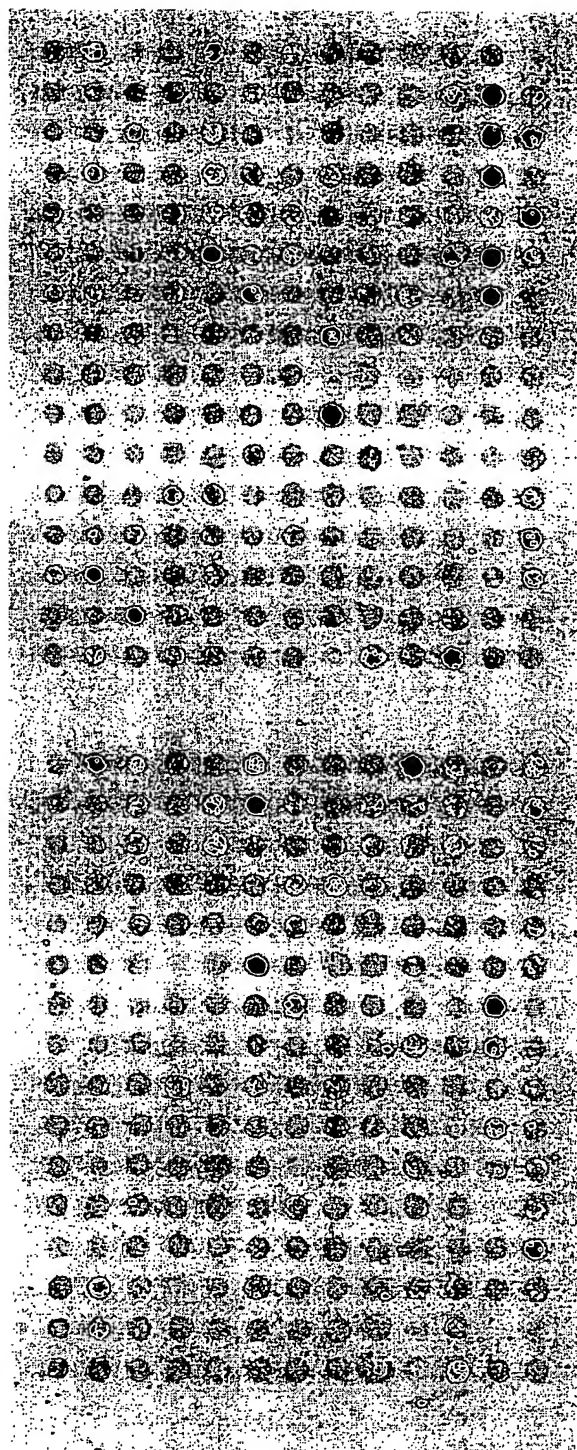


Fig. 9

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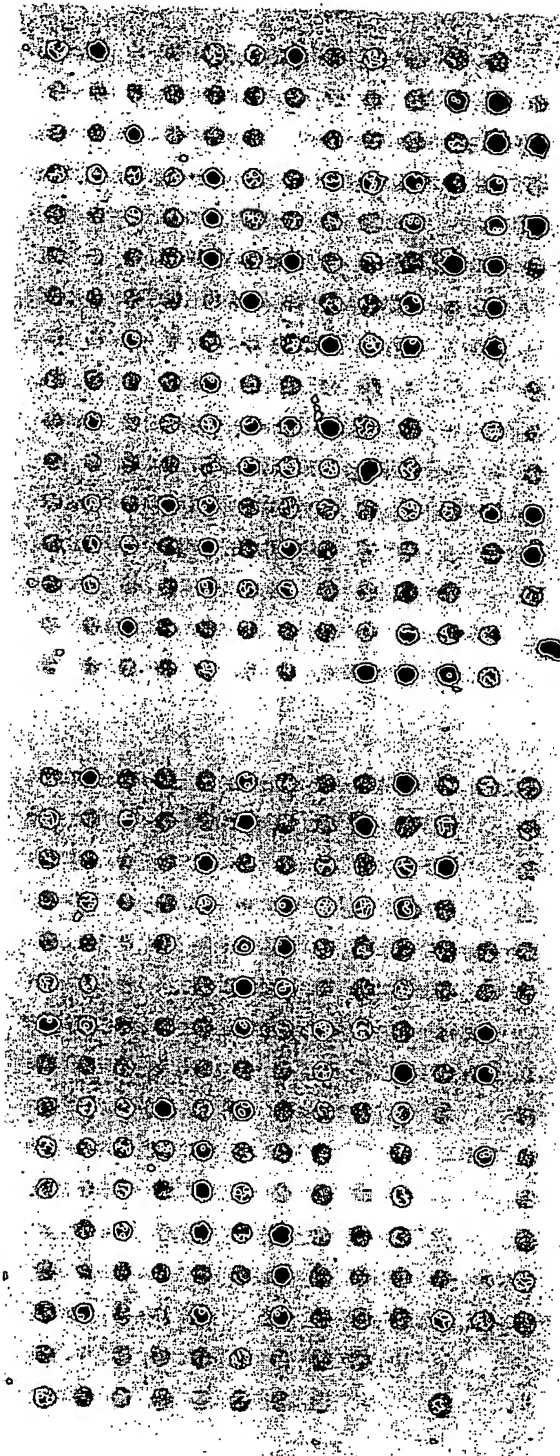


Fig. 10

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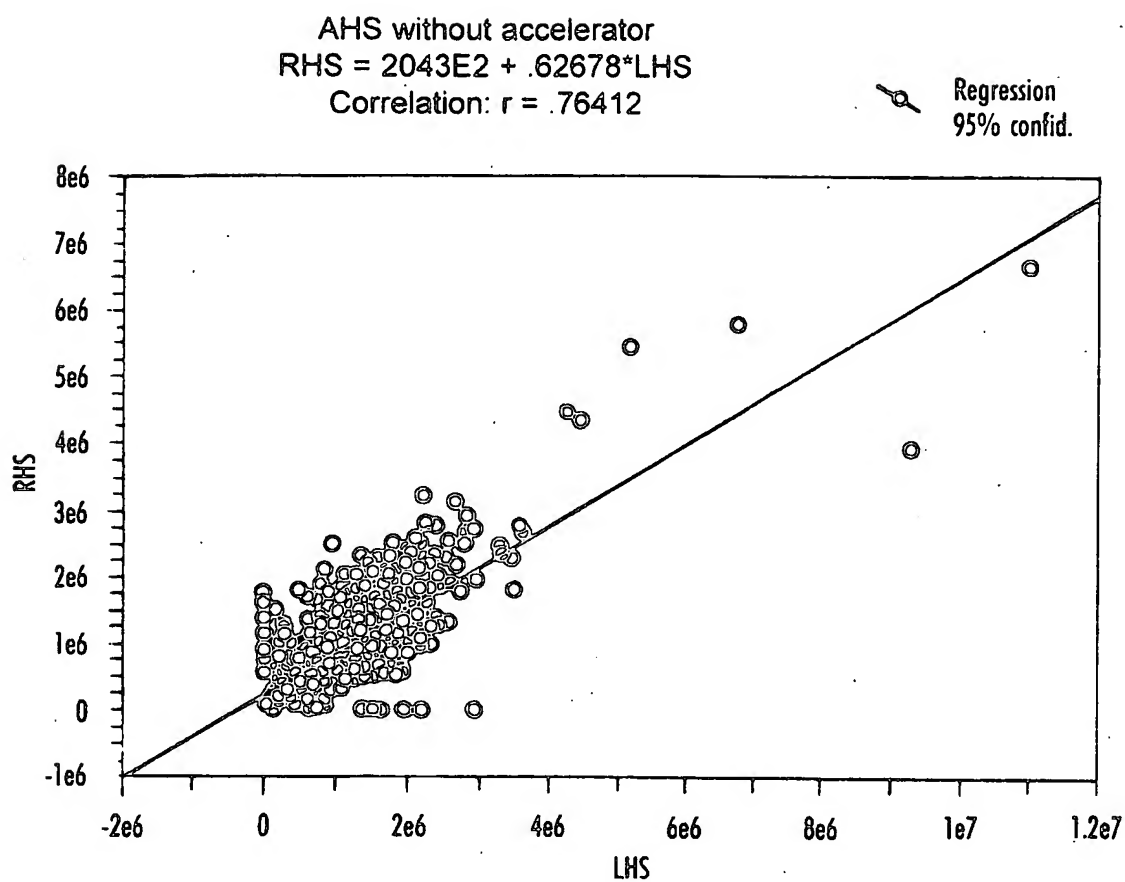


Fig. 12

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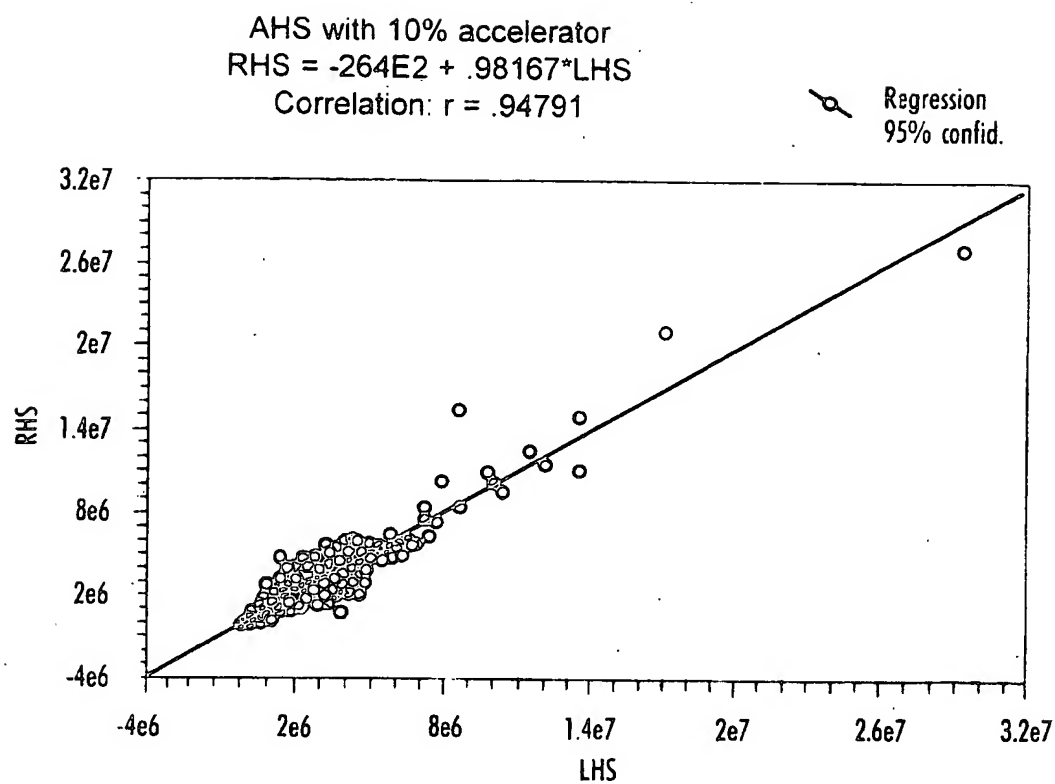


Fig. 13

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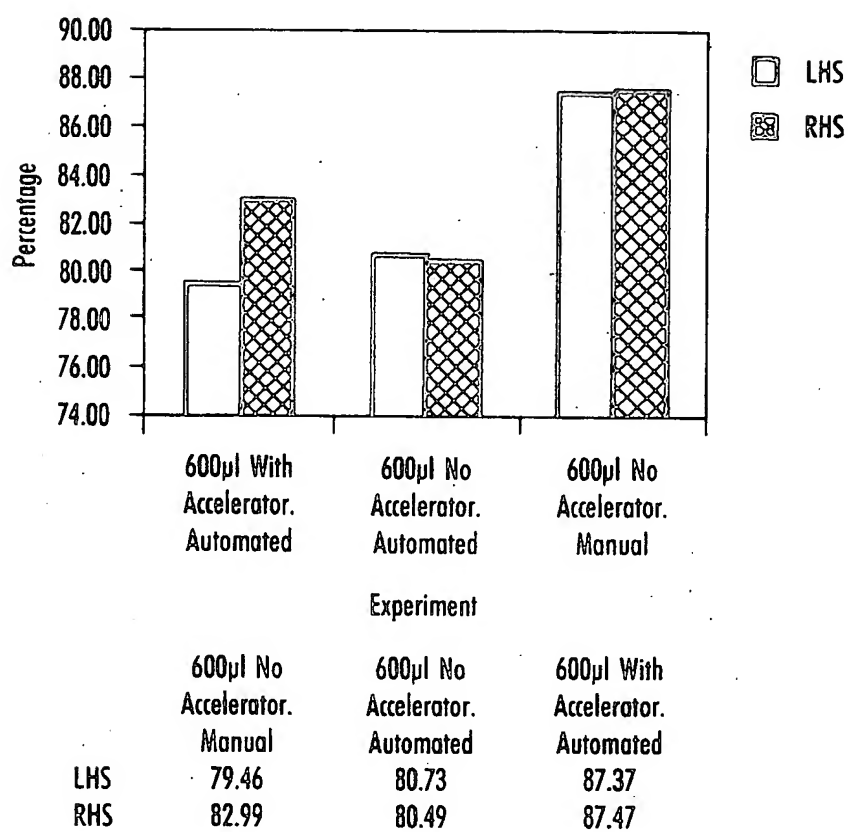
Average Solidity of Spots as a percentage  
of Maximum Solidity

Fig. 14



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Average Roundness of Spots as a Percentage  
of Maximum Solidity

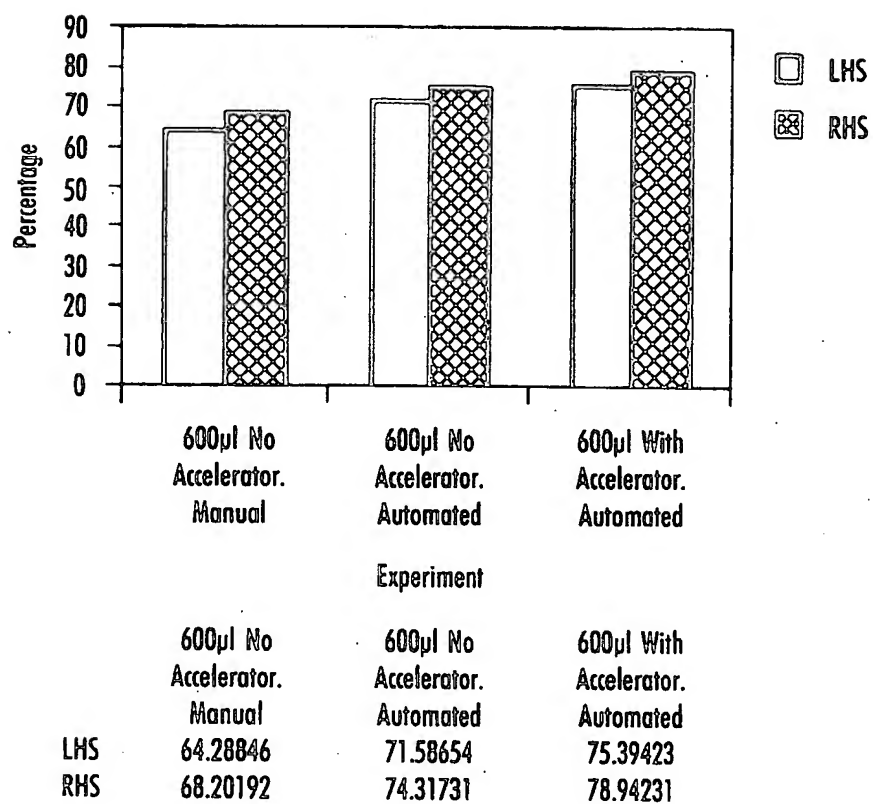


Fig. 15

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Average Circularity of Spots as a Percentage  
of Maximum Circularity

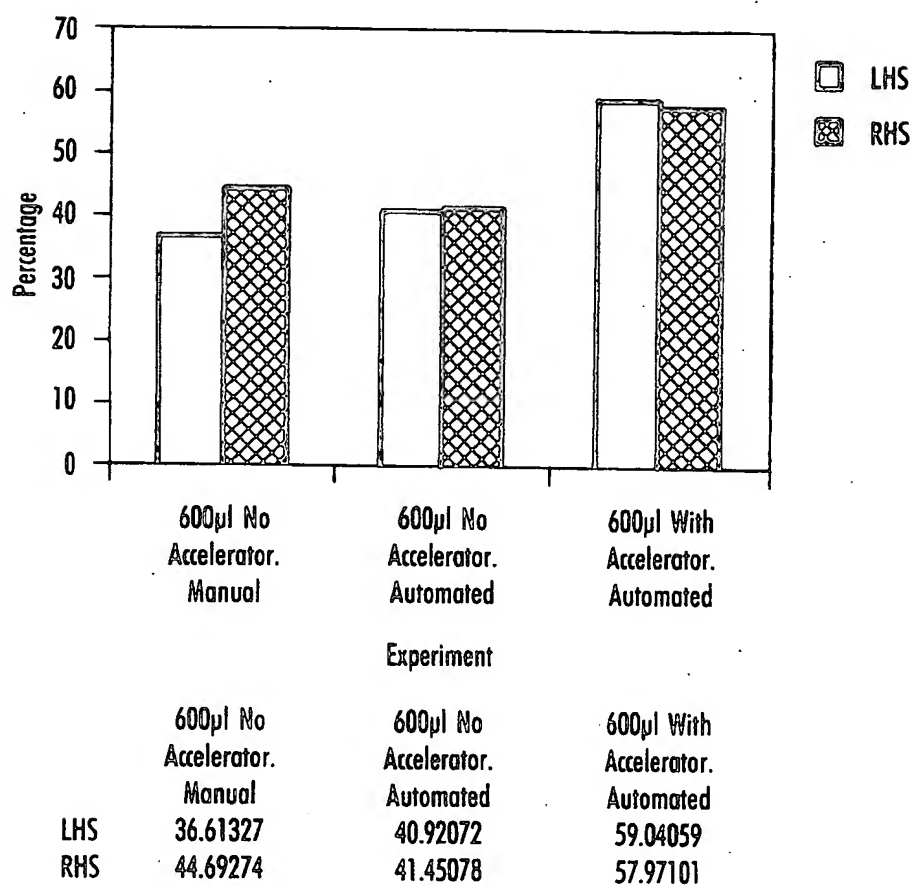


Fig. 16

# INTERNATIONAL SEARCH REPORT

Intern. Application No.

PCT/GB 00/04142

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N35/00 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N C12Q B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>US 5 451 500 A (STAPLETON MARILYN J)  19 September 1995 (1995-09-19)  column 9, line 52 -column 10, line 6  column 10, line 30 -column 10, line 39  column 10, line 57 -column 13, line 6  column 13, line 51 -column 15, line 24  column 15, line 53 -column 16, line 52  column 18, line 16 -column 19, line 14  column 21, line 25 -column 21, line 42  column 22, line 31 -column 22, line 46  column 23, line 19 -column 24, line 16  column 25, line 19 -column 26, line 26  figures 1-14</p> <p style="text-align: center;">--- -/-</p>	1,3-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

29 January 2001

Date of mailing of the international search report

02/02/2001

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Koch, A

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 00/04142

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	column 7, line 36 -column 14, line 28 figures 1-6	15
A	US 5 139 743 A (ISHIZAKA HIDEO ET AL) 18 August 1992 (1992-08-18) column 2, line 31 -column 3, line 21 column 3, line 52 -column 5, line 39 column 5, line 56 -column 6, line 8 column 9, line 50 -column 10, line 30 column 12, line 9 -column 13, line 27 column 13, line 47 -column 14, line 55 column 15, line 3 -column 16, line 68 figures 1-8	1,2,4,6, 8-10,13

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PCT/GB 00/04142

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